Tetrahedron Vol 44, No 15, pp 4713 to 4720, 1988
Printed in Great Britain

0040-4020/88 $3.00 + .00

THE SYNTHESIS OF (-)-SIRENIN, SPERM ATTRACTANT OF THE WATER MOLD ALLOMYCES MACROGYNUS†

Takeshi KITAHARA*, Akira HORIGUCHI and Kenji MORI

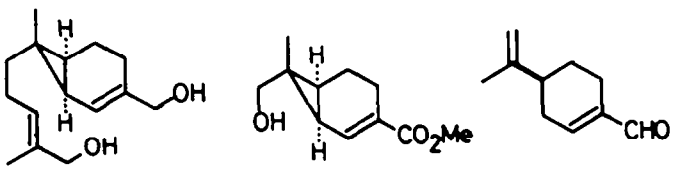
Department of Agricultural Chemistry, The University of Tokyo,
Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

(*Received in Japan* 10 *March* 1988)

Abstract -- Synthesis of (-)-sirenin **1a**, sperm attracting hormone of Allomyces macrogynus was achieved through 10 steps from (-)-perillaldehyde **3**. Formation of cyclopropane ring was executed diastereoselectively by the cyclization via intramolecular epoxide opening under carefully controlled condition.

Introduction

Sirenin **1a** is a powerful sperm attractant produced by the female gamates of the water mold Allomyces macrogynus and active at concentration of 10^{-10} M.[1] Machlis, Rapoport and co-workers described the isolation and characterization of sirenin **1a** and its 4-(4-nitrophenylazo)benzoate (NABS)[2] and then the structural elucidation.[3] The absolute configuration was rigorously assigned by the synthesis of both the enantiomers of **1a** via optical resolution and CD spectral studies of the intermediate.[3c] As **1a** has extremely interesting biological activity, many efforts have been devoted to its synthesis.[3c,4] Most of them, however, were the synthesis of (±)-**1a** except for one by Rapoport et al.[3c]



1a **2a** **3**

Fig.1

In connection with our continuing project on the chiral syntheses of microbial bioregulators, such as hormones, sporogenic substances, etc.[5], we became interested in synthesizing the natural enantiomer of sirenin (-)-**1a** with extremely high optical purity. We wish to describe here the synthesis of (-)-**1a** starting from (-)-perillaldehyde **3** via the cyclopropylcarbinol **2a**. Although Hortmann and Ong reported the preparation of **2a** from **3**,[6] there was some ambiguity about the optical purity of **2a** and they did not succeeded in converting **2a** into sirenin **1a**. We investigated carefully on this sequence, and obtained several new findings which could lead to extra pure (-)-sirenin.

†Synthetic Microbial Chemistry Part 19. Part 18: see T. Kitahara, H. Kurata and K. Mori, Tetrahedron in press. Dedicated to the Emeritus Professor M. Matsui on the occasion of his 70th birthday.

Preparation of diastereo- and enantiomerically pure cyclopropylcarbinol 2a

Commercially available (-)-perillaldehyde 3 was oxidized with $NaClO_2$[7] to give (-)-perillic acid 4 as a crystalline mass in nearly quantitative yield. Hortmann used aq-ethanol for recrystallization of 4. We examined recrystallization from various solvent systems. Among them, n-hexane was the best choice to increase the optical purity of 4. Our sample showed higher specific rotation, $[\alpha]_D$ -104.0° (c=4.0, EtOH) than the reported value[6], $[\alpha]_D$ -97.7° (EtOH). Pure 4 was transformed to a diastereomeric mixture of epoxides 6a,b in 85.2% yield in almost the same manner as reported.[6] The ratio of 6a and 6b was rigorously determined by HPLC as 1:1 (Lit.[6] 3:2). Treatment of 6a,b with prenylmagnesium chloride in the presence of CuI[8] at -30~-40°C did not give the epoxide-opening product but only undesired adduct 7 derived by 1,2-addition of Grignard reagent to ester carbonyl. So, we abandoned the introduction of side chain to 6a,b and turned to prepare cyclopropane ring earlier.

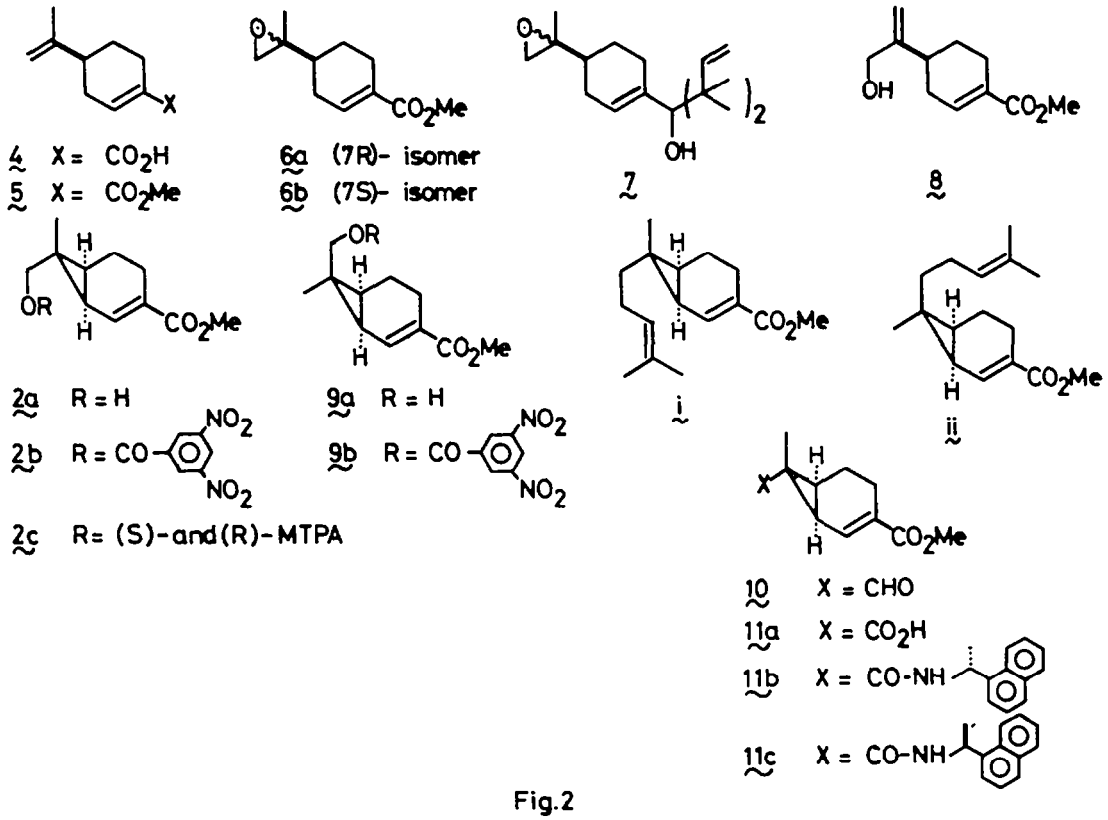


Fig.2

Base catalyzed cyclization with internal epoxide opening was carefully investigated. Using $LiN(TMS)_2$ as base, reaction proceeded at room temperature to give a mixture of the desired cyclopropylcarbinol 2a and 9a (small amount) and rearranged allylic alcohol 8 (25%). While, treatment of 6a,b with NaH in refluxing DME gave various ratios of a mixture of 2a and 9a by changing reaction period as shown in Table I.

Contrary to Hortmann's result,[6] diastereoselective cyclopropanation became possible by controlling reaction period. The desired alcohol 2a with more than 96% purity was obtained in 53% yield based on the unrecovered 6a,b (45% efficiency) under optimized condition. Stereochemistry of the products was determined by comparing the ^1H NMR data of 2a and 9a with those of i and ii in Rapoport's synthesis.[4] Thus, singlet methyl signals at δ 0.92 (major) and 1.10 (minor) were assigned to endo-methyl of 2a and exo-methyl of 9a

respectively. Twice recrystallization of the corresponding 3,5-dinitrobenzoate **2b** from n-hexane-ethyl acetate, ether-ethyl acetate and subsequent methanolysis gave 100% diastereomerically pure **2a** in 41% recovery yield from 97% pure **2a**. Specific rotation of **2a**, $[\alpha]_D$ +88.0° ($CHCl_3$), was almost 50% higher than reported,[6] $[\alpha]_D$ +60.4° ($CHCl_3$). The cyclopropylcarbinol **2a** was converted to the acid **11a** via the aldehyde **10** by PCC-MS 3A oxidation[9] and successive $NaClO_2$ oxidation[7] to make an analytical sample. The acid **11a** was transformed into (R)- and (S)-1-naphthylethylamide, **11b** and **11c**. HPLC analysis showed that **11a** (=**2a** and **10**) was practically 96% e.e. With optically pure **2a** and **10** in hand, we proceeded to introduce the side chain.

Table I

Entry	Reaction period (min)	Ratio of **2a/9a**[b]	Ratio of recovered **6a/6b**	Yield (%)[a]
a	165	90/10	50/50	46 (59)
b	150	97/3	40/60	45 (53)
c	140	96.2/3.8	37/63	-
d	110	96.4/3.6	40/60	30 (58)

[a]Yield in parentheses: based on the unrecovered epoxide **6**
[b]Monitored by HPLC

Attempt to one-step extension of the side chain

Horner-Wittig type reaction of **10** with the anion of diethyl (E)-3-methoxycarbonyl-2-butenylphosphonate **12b** gave acid-sensitive triene-ester **13** in 58% yield. (Preparation of **12b** from pyruvic acid via pure methyl (E)-4-bromo-2-methyl-2-butenoate **12a** is described in

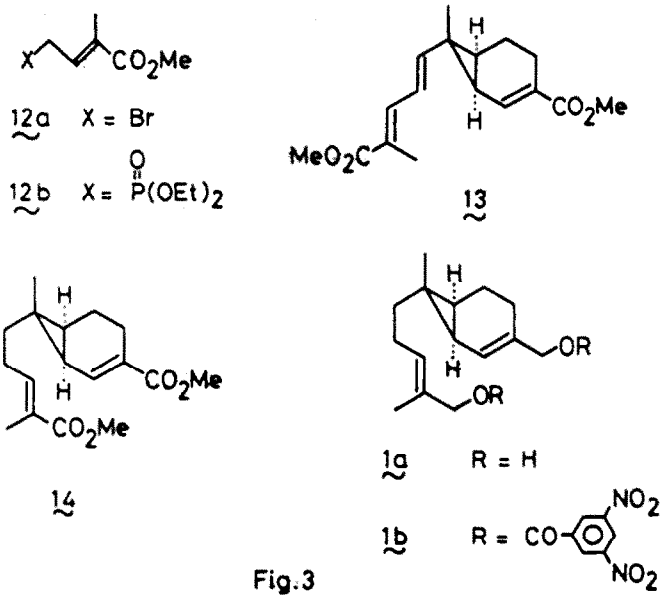
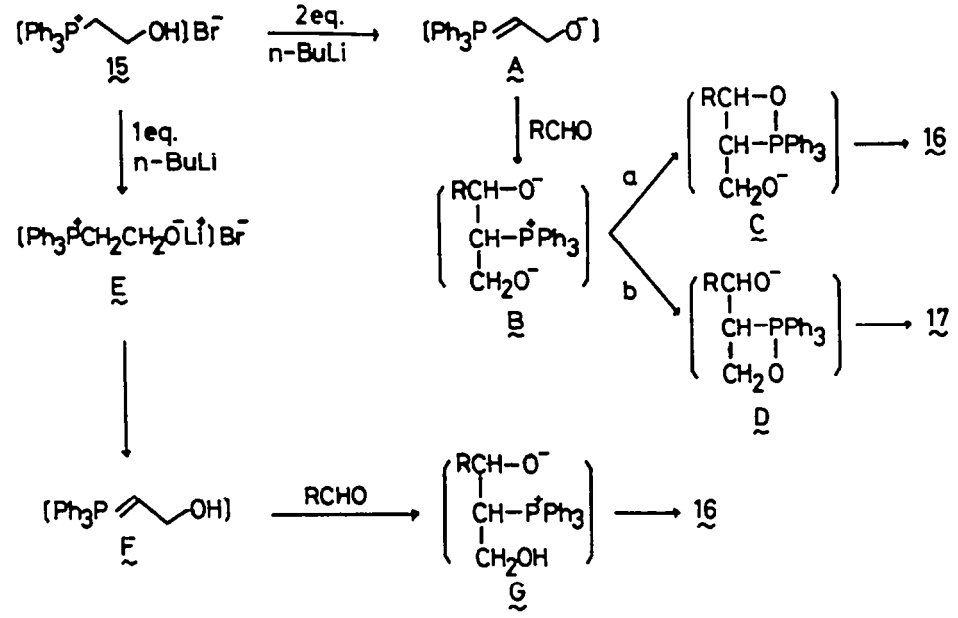


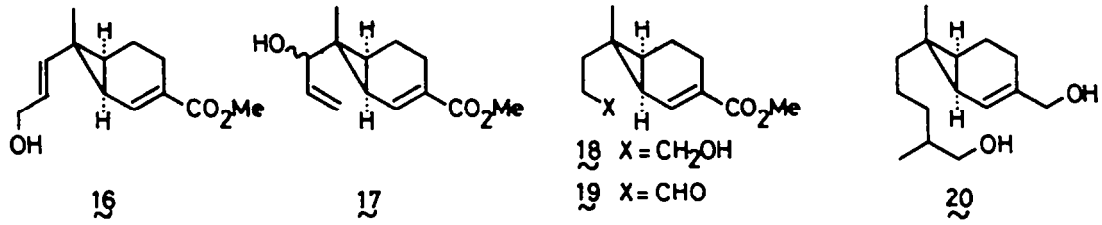
Fig.3

detail in experimental section). Many attempts for the regioselective reduction to afford the diene 14 were examined including catalytic hydrogenation over Pd-C, Pd-$BaSO_4$, Pd-$CaCO_3$, PtO_2, Rh-Al_2O_3 and $(Ph_3P)_3RhCl$ and diimide reduction.[10] Most of them gave decomposed products by reductive or prototropic cleavage of cyclopropane ring. Reduction with Pd-$BaSO_4$ gave better result and 14 was obtained as the major product. Reduction of crude 14 with $LiAlH_4$, however, gave the mixture composed of several products containing sirenin 1a. HPLC analysis of the corresponding bis-3,5-dinitrobenzoate 1b clarified that the product contained at least 5 components and was difficult to be purified. Thus, our alternative choice was stepwise introduction of the side chain.

Stepwise extension of the side chain and the synthesis of (-)-sirenin

The Wittig reaction of 10 with the phosphorane A prepared from 2-hydroxyethyltriphenylphosphonium bromide 15[11] with 2 eq. of n-BuLi gave the desired (E)-allyl alcohol 16 and substantial amount of the isomer 17, because initially formed betain B could cyclize either way to give two oxaphosphetanes, C and D, decomposing to give 16 and 17 respectively. In order to prevent this side reaction, OH group of 15 should be protected or remain intact during the formation of phosphorane. Fortunately, we succeeded in preparing 2-hydroxyethylidenetriphenylphosphorane F *in situ* from 15 using 1 eq. of n-BuLi in cold DME under salt-free condition.[11] Possible mechanism is: Initially formed lithium alkoxide E deprotonated from the carbon adjacent to phosphorus atom internally to give F





16 17 18 X = CH_2OH 19 X = CHO 20

Fig.4

with the precipitation of LiBr out of DME solution. Addition of aldehyde yields betaine G which cyclized to give single oxaphosphetane affording only **16**. The Wittig reaction of **8** with this phosporane **F** gave **16** in 65% yield with small amount of **2a** probably yielded by disproportionation. As expected, none of the isomer **17** was detected in the products. Hydrogenation of **16** over PtO_2 was followed by PCC-MS 3A oxidation[9] to give the aldehyde **19** (52%). Horner-Wittig reaction of **19** with the anion of methyl 2-diethylphosphonopropanoate gave the (E)-diene diester **14** in 78% yield along with easily separable minor (Z)-isomer (10%). Reduction of **14** with $LiAlH_4$ gave a mixture of **1a** and its dihydroderivative **20**. Using DIBAL-H suppress the contamination of **20** to give almost pure **1a**. Crude sirenin **1a** was converted to bis-3,5-dinitrobenzoate **1b** and recrystallized from isopropyl ether-DME (5:1) to give 100% analytically pure (-)-**1b**, m.p. 94.5-96°C, in 71% recovery yield from **1a**. Mild alkaline hydrolysis quantitatively yielded (-)-sirenin **1a**, $[\alpha]_D$ -44.6° (c=1.0, $CHCl_3$), whose spectral data were completely superimposable with those reported.[3]

In conclusion, the synthesis of optically pure (-)-sirenin was accomplished through 10 steps starting from (-)-perillaldehyde via diastereoselective cyclopropanation under controlled reaction condition.

EXPERIMENTAL

All b.ps and m.ps were uncorrected. IR spectra were measured as films for oils or as KBr discs for solids on a Jasco IRA-102 spectrometer. ^1H NMR spectra were recorded with TMS as an internal standard at 60 MHz on a Hitachi R-24A spectrometer or at 100 MHz on a JEOL JNM FX-100 spectrometer or at 400 MHz on a JEOL JNM GX-400 spectrometer. ^{13}C NMR spectra were measured with TMS as an internal standard as $CDCl_3$ soln at 25 MHz on a Jeol JNM FX-100 spectrometer or at 100 MHz on a JEOL JNM GX-400 spectrometer. Optical rotations were measured on a Jasco DIP 140 polarimeter. Mass spectra were recorded on a JEOL DX-303 spectrometer or a Hitachi RMU-6M spectrometer at 70 eV. Merck Kieselgel 60 (particle size 0.063-0.200 mm) was used for SiO_2 column chromatography. HPLC analyses were performed on Nucleosil 50-5 (25 cm x 4.6 mm) as a column by the detection at 254 nm unless otherwise stated.

(S)-(-)-4-(2'-Isopropenyl)-1-cyclohexenecarboxylic acid [(S)-perillic acid] **4**. A soln of $NaClO_2$ (330 g, 3.64 mol) and NaH_2PO_4 (470 g) in water (1300 ml) was added dropwise to a mixture of perillaldehyde (150 g, 1.00 mol), 2-methyl-2-butene (525 ml) and tert-BuOH (1000 ml) with ice-cooling. The mixture was stirred for 2 h at room temp and the organic layer was separated. The aq layer was extracted with ether. The combined organic soln was washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo to give crude **4**. This was recrystallized twice from n-hexane to give 81.2 g (48.9%) of **4**, m.p. 130-131°C (lit.[6] m.p. 130-132°C); $[\alpha]_D$-104° (c=4.00, EtOH) ‹lit.[6] $[\alpha]_{578}$ -97.7° (c=4, EtOH)›. Its IR and NMR spectra were identical with those reported previously.[6]

Methyl (S)-(-)-4-(2'-isopropenyl)-1-cyclohexenecarboxylate [methyl (S)-perillate] **5**. The acid **4** (34.2 g, 0.21 mol) was methylated with diazomethane in ether to give 35.5 g (96%) of **5**, b.p. 86°C/0.42 Torr (lit.[6] b.p. 81°C/0.28 Torr); $n_D^{24.5}$ 1.4855; $[\alpha]_D^{24}$ -100° (c=0.97, EtOH). Its IR and NMR spectra were identical with those reported previously.[6]

Methyl (4S,1'RS)-4-(1',2'-epoxypropyl)-1-cyclohexenecarboxylate **6a+6b**. According to the reported procedure,[6] ester **5** (35.5 g, 0.20 mol) was epoxidized with MCPBA in CH_2Cl_2 to give a crude epoxide. This was purified by SiO_2 chromatography [250 g, n-hexane-EtOAc (3:1)] to give 23.9 g (85%) of epoxide, m.p. 37-39°C. This was revealed to be a 1:1 mixture of **6a** and **6b** by HPLC analysis. HPLC [n-hexane-THF (20:1), 1.0 ml/min] Rt 17.0 min (**6a**, 50.1%), 17.5 min (**6b**, 49.9%). IR and NMR spectra of this mixture were identical with those reported previously.[6]

Methyl (1S,6S,7R)-(+)-7-hydroxymethyl-7-methylbicyclo[4.1.0]hept-2-ene-3-carboxylate **2a** and its (7S)-isomer **9a**. To a suspension of NaH (60% in mineral oil, 6.1 g, 0.15 mol) in DME (50 ml) was added dropwise a soln of **2a+9a** (15 g, 77 mmol) in DME (50 ml) under Ar. The mixture was stirred and heated under reflux for 150 min. After cooling, excess NaH was destroyed with MeOH and the mixture was poured into ice-water. This was adjusted to pH 6 with AcOH and extracted with ether. The extract was washed with sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by SiO_2 chromatography [200 g, n-hexane-EtOAc (4:1-2:3)] to give recovered epoxide (2.8 g) and 6.0 g (45%, 53% based on the consumed epoxide) of the product. This was revealed to be a 97:3 mixture of **2a** and **9a** by HPLC analysis. HPLC [n-hexane-THF (5:1), 1.0 ml/min] Rt 26.2 min (**2a**, 97%), 21.6 min (**9a**, 3.0%). Recovered epoxide was a 4:6 mixture of **6a** and **6b** by HPLC analysis as described above.

The mixture of **2a+9a** (97:3, 3.86 g, 19.7 mmol) was converted to the corresponding 3,5-dinitrobenzoates according to the reported procedure.[6] The product was recrystallized from n-hexane-EtOAc-benzene (30:9:2) and further from ether-EtOAc (15:1) to give 3.13 g (41%) of pure **2b**, m.p. 96-97°C (lit.[6] m.p. 96-98°C); $[\alpha]_D^{26}$ +7.4° (c=9.7, $CHCl_3$) ‹lit.[6] $[\alpha]_D$ +6.0°

(c=9.7, $CHCl_3$)›. Its IR and NMR spectra were identical with those reported previously.[6] HPLC [n-hexane-THF (10:1), 1.0 ml/min] crude **2b**+**9b**: Rt 18.3 min (**2b**, 98.9%), 14.3 min (**9b**, 1.1%); purified **2b**: Rt 18.3 min (100%).

The purified 3,5-dinitrobenzoate **2b** (3.62 g, 9.28 mmol) was dissolved in a mixture of MeOH (30 ml) and $CHCl_3$ (30 ml). To this was added solid K_2CO_3 (1.50 g) and the mixture was stirred for 5 h at room temp. The mixture was filtered and the filtrate was concentrated in vacuo. The residue was purified by SiO_2 chromatography [100 g, n-hexane-EtOAc (7:3-2:3)] to give 1.73 g (95%) of **2a**, n_D^{22} 1.5266; $[\alpha]_D^{22}$ +88.0° (c=8.86, $CHCl_3$) ‹lit.[6] $[\alpha]_D$ +60.4° (c=8.5, $CHCl_3$)›. Its IR and NMR spectra were identical with those reported previously.[6]

Methyl (1S,6S,7R)-(-)-7-formyl-7-methylbicyclo[4.1.0]hept-2-ene-3-carboxylate **10**. To a suspension of PCC (3.3 g, 15.3 mmol) and MS 3A (2 g) in CH_2Cl_2 (40 ml) was added dropwise a soln of **2a** (1.73 g, 8.92 mmol) in CH_2Cl_2 (10 ml) with ice-cooling. The mixture was stirred for 1 h 20 min at room temp. After diluting with ether and adding Florisil, the mixture was stirred for 15 min and filtered through Florisil. The filtrate was concentrated in vacuo to give a crude product (1.10 g). This was purified by SiO_2 chromatography [50 g, n-hexane-EtOAc (9:1-7:3)] to give 0.95 g (80%) of **10**, n_D^{25} 1.5248; $[\alpha]_D^{25}$ -17.0° (c=1.29, MeOH); νmax (film) 3020 (w), 2975 (m), 2870 (w), 2750 (m), 1710 (s), 1640 (s), 1440 (s), 1380 (w), 1360 (w), 1315 (w), 1275 (s), 1240 (s), 1180 (m), 1120 (m), 1100 (m), 1060 (m), 1030 (w), 1000 (m), 975 (w), 950 (m), 910 (m), 840 (m), 800 (w), 760 (m), 740 (m) cm^{-1}; δ (100 MHz, $CDCl_3$) 1.17 (3H, s), 1.80-2.65 (6H, m), 3.78 (3H, s), 7.08 (1H, br.d, J=5.2 Hz), 8.90 (1H, s). (Found: C, 68.06; H, 7.20. Calc for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27%).

A small portion of **10** was converted to the corresponding carboxylic acid **11a** by using $NaClO_2$ (see the preparation of **5**). The crude product was treated with (R)-naphthylethylamine and DCC in CH_2Cl_2. The product was isolated in a usual work-up procedure and followed by prepavative TLC to give an amide **11b**. In the same manner, amide **11c** was prepared from the carboxylic acid **11a** and (S)-naphthylethylamine. HPLC [n-hexane-THF (10:1), 1.0 ml/min] **11b**: Rt 49.3 min (98%), 44.8 min (2%); **11c**: Rt 49.8 min (2%), 44.7 min (98%). Therefore the optical purity of **10** was determined to be 96% e.e.

Diethyl (E)-3-methoxycarbonyl-2-butenylphosphonate **12b**. A solution of vinylmagnesium bromide was prepared from Mg (14 g, 0.57 g atom) and a soln of vinyl bromide in THF (2.5 N, 230 ml, 0.57 mol). To a solution of pyruvic acid (25 g, 0.28 mol) in THF (250 ml) was added dropwise the Grignard reagent as above between -10°C and -5°C under Ar. The reaction temp was gradually raised to 20°C and the mixture was slightly acidified with 6 N HCl. The organic layer was separated, and the aq layer was saturated with NaCl and extracted with ether. The combined organic soln was washed twice with brine, dried ($MgSO_4$) and concentrated in vacuo below 20°C. This was treated with diazomethane in ether to give the corresponding methyl ester. This was purified by SiO_2 chromatography [100 g, n-hexane-ether (65:35)]. This hydroxy ester was stirred with PBr_3 (50 g, 0.19 mol) and pyridine (7 g, 88 mmol) in ether (100 ml) with ice-cooling. The mixture was poured into water and extracted with ether. The ether soln was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by SiO_2 chromatography [200 g, n-hexane-ether (7:3)] to give crude bromide **12a**. νmax 3005 (m), 2960 (m), 1715 (s), 1640 (m), 1440 (s), 1390 (m), 1350 (m), 1270 (s), 1195 (s), 1160 (s), 1100 (s), 1080 (m), 1010 (m), 940 (m), 900 (w), 810 (m), 750 (s) cm^{-1}; δ (60 MHz, CCl_4) 1.91 (3H, d, J=2.0 Hz), 3.71 (3H, s), 3.97 (2H, d, J=8.0 Hz), 6.88 (1H, qt, J=2.0 Hz and 8.0 Hz).

A soln of **12a** in triethyl phosphite (50 ml) was stirred and heated at 120°C for 2 h. Excess triethyl phosphite was removed at 20 Torr. The residue was distilled to give 24.7 g (35% from pyruvic acid) of **12b**, b.p. 111°C/1 Torr; n_D^{21} 1.4516; νmax 2800 (m), 1790 (m), 1750 (m), 1720 (s), 1635 (m), 1440 (m), 1390 (w), 1370 (w), 1350 (w), 1260 (s), 1220 (s), 1200 (m), 1165 (m), 1100 (s), 1050 (s), 1030 (s), 965 (s), 860 (w), 820 (m), 780 (w), 750 (m), 710 (w) cm^{-1}; δ (60 MHz, CCl_4) 1.31 (6H, t, J=8.0 Hz), 1.82 (3H, dd, J=1.5 Hz and 5.0 Hz), 2.61 (2H, q, J=8.0 Hz), 3.70 (3H, s), 4.01 (4H, m), 6.62 (1H, dtq, J=8.0 Hz, 8.0 Hz and 1.5 Hz).

Methyl (1S,6S,7S,1'E,3'E)-7-(4'-methoxycarbonyl-1',3'-pentadienyl)-7-methylbicyclo[4.1.0]hept-2-ene-3-carboxylate **13**. To a suspension of NaH (60% in mineral oil, 376 mg, 9.4 mmol) in THF (15 ml) was added a soln of **12b** (2.3 g, 9.4 mmol) in THF (5 ml) with ice-cooling under Ar. The mixture was further stirred for 2 h with ice-cooling. In another flask, a soln of **10** (733 mg, 3.8 mmol) in THF (5 ml) was cooled to -14°C under Ar. To this was added the Wittig reagent as above and the mixture was stirred at -15°C for 1.5 h. The reaction was quenched with water and the mixture was extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by alumina chromaography [Woelm neutral alumina, containing 2.5% water; 136 g, n-hexane-EtOAc (95.5-85:15)] to give 500 mg of **13**, 90 mg of degraded product and 150 mg of the mixture of products. **13**: νmax 2960 (m), 1710 (s), 1630 (s), 1440 (s), 1380 (m), 1360 (m), 1270 (s), 1230 (s), 1190 (w), 1090 (s), 1050 (s), 970 (m), 940 (w), 900 (w), 750 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 1.20 (3H, s), 1.96 (3H, d, J=1.5 Hz), 1.42-2.61 (6H, m), 3.76 (3H, s), 5.73 (1H, d, J=15.0 Hz), 6.30 (1H, dd, J=10.0 Hz and 15.0 Hz), 7.14 (1H, d, J=5.0 Hz), 7.16 (1H, dd, J=1.5 Hz and 10.0 Hz). This was employed in the next step without further purification.

Transformation of **13** to **14** (General procedure). Catalyst and solvent was put into reaction flask and the atmosphere was purged with H_2. After the substrate **13** was introduced with gentle stirring, the mixture was vigorously stirred. In the case of Wilkinson's catalyst, the reaction mixture was stirred with Florisil and filtered through Florisil for product isolation. By using other catalysts, the reaction mixture was directly filtered through Celite for product isolation. In each case, the residue was concentrated in vacuo to give the crude product containing **14**.

2-Hydroxyethyltriphenylphosphonium bromide **15**. To a soln of triphenylphosphine (25.2 g, 96 mmol) in benzene (200 ml) was added 2-bromoethanol (10 g, 80 mmol) and the mixture was stirred and heated to reflux overnight. After cooling, the mixture was filtered. The crystals on the filter was washed with benzene to give 30.4 g (86%) of **15**. This was dried in vacuo at 110°C for 19 h for further use.

Methyl (1S,6S,7S,1'E)-(-)-7-(3'-hydroxy-1'-propenyl)-7-methylbicyclo[4.1.0]hept-2-ene-3-carboxylate **16**. To a soln of phos-

phonium bromide **15** (7.5 g, 19.4 mmol) in DME (50 ml) was added dropwise n-BuLi in n-hexane (1.6 N, 9.5 ml, 15.2 mmol) at -10°C under Ar. The mixture was stirred at -10°C for 30 min and left to stand for 30 min to precipitate inorganic salt. In another flask, a soln of **10** (713.5 mg, 3.68 mmol) in DME (10 ml) was cooled to -10°C under Ar. To this was added the supernatant of Wittig reagent as above (ca. 50 ml) and the reaction temp was gradually raised to room temp. The reaction was quenched with water, and the mixture was extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by SiO_2 chromatography [50 g, n-hexane-EtOAc (3:1-2:3)] to give 530.4 mg (65%) of **16**, n_D^{26} 1.5463; $[\alpha]_D^{26}$ -123° (c=1.16, MeOH); νmax 3400 (s), 3000 (w), 2950 (m), 2860 (w), 1710 (s), 1660 (w), 1640 (s), 1440 (s), 1385 (w), 1275 (s), 1240 (s), 1120 (w), 1090 (m), 1050 (m), 1020 (w), 990 (m), 970 (m), 905 (w), 935 (w), 745 (m) cm^{-1}; δ (100 MHz, $CDCl_3$) 1.05 (3H, s), 1.10-2.58 (6H, m), 3.75 (3H, s), 4.13 (3H, br.t, J=5.0 Hz, D_2O exchangeable, d, J=6.0 Hz), 5.37 (1H, d, J=16.0 Hz), 5.60 (1H, dt, J=17.0 Hz and 6.0 Hz), 7.16 (1H, dd, J=5.0 Hz and 2.0 Hz). HRMS m/z Found:222.1286. Calc for $C_{13}H_{18}O_3$: 222.1256.

Methyl (1S,6S,7R)-(+)-7-(3'-hydroxypropyl)-7-methylbicyclo[4.1.0]hept-2-ene-3-carboxylate **18**. A mixture of PtO_2 (20 mg) and EtOAc (7 ml) was vigorously stirred under H_2 and cooled to 5°C. To this was added dropwise a soln of **16** (738.7 mg, 3.33 mmol) in EtOAc (3 ml) and the mixture was stirred at 5-10°C for 1 h. After the catalyst was filtered off, the filtrate was concentrated in vacuo to give 730.5 mg (98%) of **18**, $n_D^{24.5}$ 1.5072; $[\alpha]_D^{24}$ +18.3° (c=1.01, MeOH); νmax 3450 (m), 2950 (s), 2900 (m), 1710 (s), 1640 (m), 1440 (m), 1380 (w), 1270 (s), 1240 (s), 1210 (w), 1090 (w), 1060 (m), 980 (w), 910 (w), 840 (w), 800 (w), 750 (w) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.90 (3H, s), 0.93-2.60 (11H, m), 3.64 (2H, t, J=6.0 Hz), 3.73 (3H, s), 7.23 (1H, dm, J=6.0 Hz). HRMS m/z Found: 224.1380. Calc for $C_{13}H_{20}O_3$: 224.1413.

Methyl (1S,6S,7R)-(+)-7-(2'-formylethyl)-7-methylbicyclo[4.1.0]hept-2-ene-3-carboxylate **19**. In the similar manner as described for the preparation of **10**, alcohol **18** (503 mg, 2.25 mmol) was oxidized with PCC in CH_2Cl_2 to give 260.3 mg (52%) of **19**, $n_D^{24.5}$ 1.5064; $[\alpha]_D^{25.5}$ +41.1° (c=1.12, MeOH); νmax 3025 (m), 2950 (m), 2750 (m), 1720 (s), 1640 (s), 1440 (s), 1410 (s), 1390 (m), 1270 (s), 1240 (s), 1210 (w), 1190 (w), 1130 (w), 1100 (s), 1060 (s), 910 (s), 840 (w), 800 (w), 750 (m) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.85 (3H, s), 1.00-2.67 (10H, m), 3.74 (3H, s), 7.20 (1H, br.d, J=5.0 Hz), 9.80 (1H, t, J=1.8 Hz). (Found: C, 69.76; H, 8.16. Calc for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16%).

Methyl (1S,6S,7R,3'E)-(+)-7-(4'-ethoxycarbonyl-3'-pentenyl)-7-methylbicyclo[4.1.0]hept-2-ene-3-carboxylate **14**. According to the reported procedure,[4d] aldehyde **19** (213 mg, 0.96 mmol) was converted to the diesters **14** and the corresponding (3'Z)-isomer. Crude product was purified twice by SiO_2 chromatography [10 g, n-hexane-EtOAc (19:1-17:3)] to give 227.5 mg (78%) of **14** and 32.7 mg (11%) of the corresponding (3'Z)-isomer. **14**: n_D^{25} 1.5041; $[\alpha]_D^{26}$ +45.5° (c=1.03, MeOH). Its IR and NMR spectra were identical with those reported previously.[4d] (Found: C, 70.18; H, 8.35. Calc for $C_{18}H_{26}O_4$: C, 70.56; H, 8.55%).

(-)-Sirenin **1a**. To a soln of **14** (143.4 mg, 0.47 mmol) in toluene (4 ml) was added dropwise DIBAL-H in toluene (1 M, 1.5 ml, 1.5 mmol) at -5°C. The reaction temp was raised to 0-2°C, and to the mixture was added DIBAL-H soln (1.0 ml). After stirring at 0-2°C for 10 min, MeOH (3 ml) was added to the mixture and the resulting mixture was stirred for 40 min. Then it was filtered through Celite and alumina and the filtrate was concentrated in vacuo to give a crude product. This was purified by SiO_2 preparative HPLC [YMC A-024 silica gel, 10 mm x 300 mm, n-hexane-EtOAc (3:2), 3.5 ml/min, detected by refractive index (Shodex SE-51)] Rt 20 min] to give 70.5 mg of crude (-)-Sirenin (64%). This was further purified as follows.

(-)-Sirenin **1a** (37 mg, 0.17 mmol) was converted to the corresponding bis-3,5-dinitrobenzoate **1b** in a usual manner. This was purified by SiO_2 chromatography [6.5 g, n-hexane-EtOAc (97:3-17:3)] followed by recrystallization from diisopropyl ether-DME to give 69 mg (71%) of **1b**, m.p. 94.5-96°C; $[\alpha]_D^{26}$ -19.5° (c=1.00, $CHCl_3$); νmax (KBr) 3140 (m), 2950 (m), 1730 (s), 1640 (m), 1550 (s), 1460 (m), 1350 (s), 1290 (s), 1170 (s), 1080 (m), 950 (m), 920 (m), 880 (w), 860 (w), 830 (w), 780 (w), 740 (s), 730 (s) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.94 (3H, s), 0.98-2.36 (10H, m), 1.79 (3H, s), 4.85 (4H, br.s), 5.13 (1H, br.t, J=7.0 Hz), 6.08 (1H, br.d, J=4.0 Hz), 9.17 (6H, m). HPLC [n-hexane-THF (10:1), 1.0 ml/min] Rt 17.0 min (100%). (Found: C, 55.70; H, 4.62; N, 8.83. Calc for $C_{29}H_{28}O_{12}N_4$: C, 55.77; H, 4.52; N, 8.97%).

To a soln of bis-3,5-dinitrobenzoate **1b** (68 mg, 0.11 mmol) in THF (7 ml) and water (2 ml) was added N KOH (1 ml) with ice-cooling. The mixture was stirred for 3 h with ice-cooling, poured into sat $NaHCO_3$ soln and extracted three times with ether. The ether soln was washed with brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by alumina chromatography [Woelm neutral alumina, containing 2.5% water; 5 g, $CHCl_3$ (50 ml) followed by $CHCl_3$-MeOH (50:1, 50 ml)] to give 25.3 mg (98%) of **1a**, n_D^{24} 1.5210; $[\alpha]_D^{24}$ -44.6° (c=1.07, $CHCl_3$); νmax 3325 (s), 3000 (m), 2925 (s), 2850 (s), 1660 (w), 1450 (m), 1380 (m), 1350 (m), 1215 (m), 1160 (m), 1120 (m), 1060 (m), 1000 (s), 905 (w), 865 (m), 825 (m) cm^{-1}. δ (400 MHz, $CDCl_3$) 0.88 (3H, s), 0.93 (1H, ddd, J=2.5, 7.0, 8.0 Hz), 1.03 (1H, dd, J=5.0, 8.0 Hz), 1.17-1.28 (1H, m), 1.32-1.44 (2H, m), 1.46 (2H, br.s), 1.67 (3H, s), 1.69-1.92 (2H, m), 2.00-2.07 (1H, m), 2.12 (2H, dt, J=8.0, 16.0 Hz), 4.01 (4H, d, J=8.0 Hz), 5.40 (1H, tq, J=8.0, 1.8 Hz), 5.84 (1H, d, J=5.0 Hz). ^{13}C NMR (25 MHz, $CDCl_3$) δ 12.68, 13.58, 17.50, 21.66, 21.89, 23.44, 25.14, 28.88, 42.51, 67.55, 68.99, 121.43, 126.35, 134.42, 137.17. HRMS m/z Found: 236.1748. Calc for $C_{15}H_{24}O_2$: 236.1777. (Found: C, 75.88; H, 10.00. Calc for $C_{15}H_{24}O_2$: C, 76.22; H, 10.24%).

Acknowledgements -- Much thanks are due to Professor H. Rapoport for the identification of our synthetic sirenin with authentic sample. We thank T. Hasegawa Co. Ltd. for the generous gift of (-)-perillaldehyde. A part of this work was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture and by The Naito Foundation.

REFERENCES

1) a) L. Machlis, Physiol. Plant., 11, 181, 845 (1958).
   b) L. Machlis, Nature, 181, 1790 (1958).
   c) L. Machlis and E. Rawitscher-Kunkel, Intern. Rev. Cytol., 15, 97 (1963).
2) L. Machlis, W. H. Nutting, R. W. Williams and H. Rapoport, Biochemistry, 5, 2147 (1966).
3) a) L. Machlis, W. H. Nutting, M. W. Williams and H. Rapoport, J. Am. Chem. Soc., 90, 1674 (1968).
   b) W. H. Nutting, H. Rapoport and L. Machlis, J. Am. Chem. Soc., 90, 6434 (1968).
   c) J. J. Plattner and H. Rapoport, J. Am. Chem. Soc., 93, 1758 (1971).
4) a) E. J. Corey, K. Achiwa and A. Katzenellenbogen, J. Am. Chem. Soc., 91, 4318 (1969).
   b) J. J. Plattner, U. T. Bhalerao and H. Rapoport, J. Am. Chem. Soc., 91, 4933 (1969).
   c) P. A. Grieco, J. Am. Chem. Soc., 91, 5660 (1969).
   d) K. Mori and M. Matsui, Tetrahedron Lett., 4485 (1969); Tetrahedron, 26, 2801 (1970).
   e) U. T. Bhalerao, J. J. Plattner and H. Rapoport, J. Am. Chem. Soc., 92, 3429 (1970).
   f) E. J. Corey and K. Achiwa, Tetrahedron Lett., 2245 (1970).
   g) C. F. Garber, J. A. Steenkamp and H. E. Visagie, Tetrahedron Lett., 3753 (1975); S. Afr. J. Chem., 33, 21 (1980).
   h) K. Kitatani, T. Hiyama and H. Nozaki, J. Am. Chem. Soc., 98, 2362 (1976).
   i) T. Mandai, K. Hara, M. Kawada and J. Nokami, Tetrahedron Lett., 24, 1517 (1983).
   j) T. Uehara, J. Yamada, K. Ogata and T. Kato, Bull. Chem. Soc. Jpn., 58, 211 (1985).
5) a) K. Mori, Tetrahedron, 29, 3107 (1983).
   b) T. Kitahara, H. Kurata, T. Matsuoka and K. Mori, Tetrahedron, 41, 5475 (1985).
   c) K. Mori and H. Tamura, Liebigs Ann. Chem., in press.
   d) T. Kitahara, H. Kurata and K. Mori, Tetrahedron, in press.
6) A. G. Hortmann and A. Q. Ong, J. Org. Chem., 35, 4290 (1970).
7) B. S. Gal, W. E. Childers and H. W. Pinnick, Tetrahedron, 37, 2091 (1981).
8) G. Linstrumelle, R. Lorne, and H. P. Dang, Tetrahedron Lett., 4069 (1978).
9) J. Hamsoovici and H. Antonakis, J. Chem. Soc. Chem. Commun., 561 (1980).
10) K. Mori, M. Chki, A. Sato and M. Matsui, Tetrahedron, 28, 3719 (1972). and references cited therein.
11) S. Danishefsky, P. F. Schuda, T. Kitahara and S. J. Etheredge, J. Am. Chem. Soc., 99, 6066 (1977).